Null Results in Brief

Common Genetic Variants in the Vitamin D Pathway Including Genome-Wide Associated Variants Are Not Associated with Breast Cancer Risk among Chinese Women

Tsogzolmaa Dorjgochoo¹, Ryan Delahanty¹, Wei Lu², Jirong Long¹, Qiuyin Cai¹, Ying Zheng², Kai Gu², Yu-Tang Gao³, Wei Zheng¹, and Xiao Ou Shu¹

Abstract

Background: Previous studies evaluating the association of vitamin D–related genetic variants with breast cancer risk have produced inconsistent results.

Methods: We evaluated the association between breast cancer risk and 559 single-nucleotide polymorphisms (SNP) in 12 vitamin D–related genes, including 6 genes associated with circulating 25-hydroxyvitamin D [25(OH)D] level identified by recent genome-wide association studies (GWAS), using directly observed and imputed GWAS genotyping data from 2,919 breast cancer cases and 2,323 controls recruited in the Shanghai Breast Cancer Study.

Results: Of the SNPs studied, only rs12570116 in the ACADSB gene, rs4760658 in the VDR gene and rs6091822, rs8124792, and rs6097809 in the CYP24A1 gene, and rs10902845 in C10orf88 had a nominal association with breast cancer risk (P < 0.05 for all). None of these associations persisted after adjustment for multiple comparisons. The most extensively studied SNPs including rs10735810, also known as rs2228570 (Fok1, VDR), rs1544410 (Bsm1, VDR), and rs2296241 (CYP24A1), were not associated with breast cancer risk. GWAS-identified genetic variants that were associated with 25(OH)D were also not related to breast cancer risk.

Conclusions: Our data suggest that genetic polymorphisms in vitamin D–related genes do not play a major role in breast cancer risk in Chinese women.

Impact: Although our study confirms previously documented breast cancer risk factor associations, our null results suggest that common genetic variants in vitamin D genes and loci associated with control of vitamin D levels are not risk factors for breast cancer in Chinese women. Our data contribute to filling the gap in this field of research. Cancer Epidemiol Biomarkers Prev; 20(10); 2313–6. ©2011 AACR.

Introduction

In recent years, the role of vitamin D in the etiology of breast cancer has been increasingly recognized because of its importance in cell proliferation, apoptosis, and differentiation in normal and malignant tumor cells (1, 2). Numerous epidemiologic studies have suggested that vitamin D status or circulating 25-hydroxyvitamin D [25(OH)D] level (1) and common variants that affect vitamin D production and signaling may play a role in the development of breast cancer (2, 3); however, the results have been inconclusive. No epidemiologic study has yet simultaneously evaluated the association between polymorphisms in vitamin D pathway genes [25(OH)D-1-alpha hydroxylase (CYP27B1), vitamin D (3) 24-hydroxylase (CYP27A1), vitamin D 24-hydroxylase (CYP24A1), vitamin D binding protein (GC), cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4), cytochrome P450 arachidonic acid epoxygenase (CYP2J2), cytochrome P450, family 2, subfamily R, polypeptide 1 (CYP2R1), and vitamin D receptor gene (VDR); ref. 2], as well as in novel genes associated with 25(OH)D level that have been identified by recent genome-wide association studies [GWAS; NAD synthetase (NADSYN1), 7-dehydrocholesterol reductase (DHCR7), acyl-coenzyme A dehydrogenase (ACADSB), and the region of chromosome 10 harboring open-reading frame 88 (C10orf88); refs. 4, 5] and breast cancer risk. We comprehensively examined this association using data on more than 5,000 women from the Shanghai Breast Cancer GWAS (SBC-GWAS).
Materials and Methods

Study population
This study includes data from 5,242 Chinese women, aged 25 to 70 years, in the SBC-GWAS, which drew its data from women who participated in the Shanghai Breast Cancer Study (SBCS, phases I and II), a population-based case–control study. Detailed methods for the SBCS and the SBC-GWAS have been published elsewhere (6). This study was approved by all participating institutions, and participants provided written informed consent.

Single-nucleotide polymorphism genotyping, selection, and imputation
Genotyping information was generated using the Affymetrix 6.0 array as described in detail previously (6). The 8 genes (CYP27B1, CYP27A1, CYP24A1, GC, CYP3A4, CYP2J2, CYP2R1, and VDR) evaluated in this study were selected on the basis of their potential biologic role in vitamin D metabolism and signaling as determined by literature review (2, 4), as well as an informatics tool, the STRING database (version 8.3). In addition, we included genes (NADSYN1, ACADSB, DHCR7, and C10orf88) that have been associated with 25(OH)D concentration, as identified by recent GWASs (4, 5). Single-nucleotide polymorphisms (SNP) were selected within the region 10 kb upstream of the transcription start site and 10 kb downstream from the end of each gene. A total of 559 SNPs (175 directly observed and 384 imputed) in these 12 vitamin D-related genes with a minor allele frequency of 5% or more were included in the analyses.

Statistical analysis
Descriptive statistics and genome-wide analyses were conducted within each sample set and in aggregate using SAS (version 9.2) and PLINK, respectively, as described.
Results

Linkage disequilibrium was assessed by Haploview. were not corrected for multiple testing (Tables 1 and 2).

Breast cancer risk and polymorphisms under an additive genetic model, controlling for age and education.

ACADSB we analyzed, only 6 SNPs (rs12570116 in ACADSB, rs7041 in C10orf88, rs2296241 in VDR, and rs6091822, rs8124792, and rs6097809 in CYP24A1) were associated with breast cancer risk (\(P < 0.05\) for all, Table 1). However, these nominally significant associations were not significant after accounting for multiple testing. We also found no association between breast cancer risk and the most extensively studied genetic polymorphisms (2), including Fok1 (rs10735810), Bsm1 (rs1544410), Taq1 (rs731236), and Apa1 (rs7975232) in VDR and rs2296241 in CYP24A1 (data not shown). Of the 22 SNPs associated with 25(OH)D level identified by prior GWAS, none were associated with breast cancer risk in our population (Table 2). Furthermore, in analyses stratified by menopausal status, physical activity, or dietary energy intake, no polymorphisms were associated with breast cancer risk (data not shown).

Discussion

To our knowledge, this is the first comprehensive examination of common genetic variations (559 SNPs) across 12 genes related to vitamin D metabolism and signaling and breast cancer risk. Overall, vitamin D

### Table 2. GWAS-identified SNPs associated with circulating vitamin D [25(OH)D] levels and proxy-SNPs in relation to breast cancer risk in the SBC-GWAS (2,919 cases and 2,323 controls)

<table>
<thead>
<tr>
<th>GWAS SNP</th>
<th>Chr</th>
<th>Genomic position</th>
<th>Gene location or nearest gene</th>
<th>Effective allele and frequency</th>
<th>Per allele OR (95% CIs) (a)</th>
<th>(P)</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17467825</td>
<td>4</td>
<td>72824381</td>
<td>GC</td>
<td>A 0.69</td>
<td>0.98 (0.90–1.08)</td>
<td>0.78</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs2282679</td>
<td>4</td>
<td>72827247</td>
<td>GC</td>
<td>G 0.31</td>
<td>1.01 (0.92–1.10)</td>
<td>0.82</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs3755967</td>
<td>4</td>
<td>72828262</td>
<td>GC</td>
<td>T 0.31</td>
<td>1.01 (0.92–1.09)</td>
<td>0.89</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs2298850</td>
<td>4</td>
<td>72831301</td>
<td>GC</td>
<td>C 0.31</td>
<td>1.01 (0.92–1.09)</td>
<td>0.88</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs7041</td>
<td>4</td>
<td>72837198</td>
<td>GC</td>
<td>A 0.72</td>
<td>1.03 (0.94–1.13)</td>
<td>0.44</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs1155563</td>
<td>4</td>
<td>72862352</td>
<td>GC</td>
<td>C 0.40</td>
<td>1.02 (0.94–1.12)</td>
<td>0.55</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs1993116</td>
<td>11</td>
<td>14866810</td>
<td>CYP2R1</td>
<td>A 0.35</td>
<td>0.98 (0.90–1.06)</td>
<td>0.58</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs1050804</td>
<td>11</td>
<td>14868449</td>
<td>CYP2R1</td>
<td>G 0.37</td>
<td>1.00 (0.92–1.08)</td>
<td>0.98</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs1279471</td>
<td>11</td>
<td>14870151</td>
<td>CYP2R1</td>
<td>A 0.37</td>
<td>0.99 (0.91–1.07)</td>
<td>0.87</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs1074165</td>
<td>11</td>
<td>14871454</td>
<td>CYP2R1</td>
<td>A 0.35</td>
<td>0.97 (0.90–1.05)</td>
<td>0.56</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs2060793</td>
<td>11</td>
<td>14871886</td>
<td>CYP2R1</td>
<td>A 0.35</td>
<td>0.98 (0.90–1.06)</td>
<td>0.56</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs7944926</td>
<td>11</td>
<td>70843273</td>
<td>DHCR7/NADSYN1</td>
<td>A 0.54</td>
<td>0.94 (0.87–1.02)</td>
<td>0.19</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs1278587</td>
<td>11</td>
<td>70845097</td>
<td>DHCR7/NADSYN1</td>
<td>T 0.46</td>
<td>1.02 (0.94–1.10)</td>
<td>0.58</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs4499457</td>
<td>11</td>
<td>70845683</td>
<td>DHCR7/NADSYN1</td>
<td>G 0.45</td>
<td>1.01 (0.93–1.09)</td>
<td>0.79</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs1280043</td>
<td>11</td>
<td>70848651</td>
<td>DHCR7/NADSYN1</td>
<td>A 0.45</td>
<td>1.04 (0.96–1.12)</td>
<td>0.30</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs3794060</td>
<td>11</td>
<td>70865327</td>
<td>DHCR7/NADSYN1</td>
<td>C 0.54</td>
<td>0.95 (0.88–1.03)</td>
<td>0.25</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs4945008</td>
<td>11</td>
<td>70898896</td>
<td>DHCR7/NADSYN1</td>
<td>A 0.55</td>
<td>0.95 (0.88–1.02)</td>
<td>0.21</td>
<td>Imputed</td>
</tr>
<tr>
<td>rs1790349</td>
<td>11</td>
<td>70819998</td>
<td>DHCR7/NADSYN1</td>
<td>C 0.29</td>
<td>0.97 (0.89–1.06)</td>
<td>0.56</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs3829251</td>
<td>11</td>
<td>70872207</td>
<td>NADSYN1</td>
<td>A 0.30</td>
<td>0.94 (0.86–1.02)</td>
<td>0.16</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs10988193</td>
<td>11</td>
<td>70874731</td>
<td>NADSYN1</td>
<td>T 0.30</td>
<td>0.94 (0.86–1.02)</td>
<td>0.16</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs11234027</td>
<td>11</td>
<td>124786160</td>
<td>C10orf88</td>
<td>G 0.12</td>
<td>0.96 (0.85–1.08)</td>
<td>0.49</td>
<td>Genotyped</td>
</tr>
<tr>
<td>rs17104498</td>
<td>10</td>
<td>52201798</td>
<td>CYP24A1</td>
<td>C 0.10</td>
<td>1.04 (0.91–1.18)</td>
<td>0.53</td>
<td>Genotyped</td>
</tr>
</tbody>
</table>

\(a\)Adjusted for age at interview and education.

\(b\)The SUNLIGHT GWAS of 15 cohorts (United Kingdom, United States, Canada, the Netherlands, Sweden, and Finland).

\(c\)Meta-analysis of 5 GWAS within 5 cohorts (European ancestry).

Previously (6), multivariate logistic regression was used to estimate ORs and 95% CIs for associations between breast cancer risk and polymorphisms under an additive genetic model, controlling for age and education. \(P\) values were not corrected for multiple testing (Tables 1 and 2). Linkage disequilibrium was assessed by Haploview.

Results

The mean age was 50.7 years for cases and 49.6 years for controls. As compared with controls, cases were more likely to have higher educational attainment, earlier age at menarche, later age at first birth and menopause, longer reproductive span, family history of breast cancer among first-degree relatives, and a higher waist-to-hip ratio (data not shown). Of the 559 SNPs in the 12 genes that we analyzed, only 6 SNPs (rs12570116 in ACADSB, rs10902845 in C10orf88, rs4760658 in VDR, and rs6091822, rs8124792, and rs6097809 in CYP24A1) were associated with breast cancer risk (\(P < 0.05\) for all, Table 1). However, the mean age was 50.7 years for cases and 49.6 years for controls. As compared with controls, cases were more likely to have higher educational attainment, earlier age at menarche, later age at first birth and menopause, longer reproductive span, family history of breast cancer among first-degree relatives, and a higher waist-to-hip ratio (data not shown). Of the 559 SNPs in the 12 genes that we analyzed, only 6 SNPs (rs12570116 in ACADSB, rs10902845 in C10orf88, rs4760658 in VDR, and rs6091822, rs8124792, and rs6097809 in CYP24A1) were associated with breast cancer risk (\(P < 0.05\) for all, Table 1). However, these nominally significant associations were not significant after accounting for multiple testing. We also found no association between breast cancer risk and the most extensively studied genetic polymorphisms (2), including Fok1 (rs10735810), Bsm1 (rs1544410), Taq1 (rs731236), and Apa1 (rs7975232) in VDR and rs2296241 in CYP24A1 (data not shown). Of the 22 SNPs associated with 25(OH)D level identified by prior GWAS, none were associated with breast cancer risk in our population (Table 2). Furthermore, in analyses stratified by menopausal status, physical activity, or dietary energy intake, no polymorphisms were associated with breast cancer risk (data not shown).

Discussion

To our knowledge, this is the first comprehensive examination of common genetic variations (559 SNPs) across 12 genes related to vitamin D metabolism and signaling and breast cancer risk. Overall, vitamin D
pathway gene polymorphisms were not associated with breast cancer risk in our population. The suggestive association for 6 SNPs in the ACADSB, VDR, and CYP24A1 genes and C10orf88 could be due to chance and need to be further investigated in other populations.

Among the vitamin D pathway genes, VDR SNPs (Apa1: rs7975232, Bsm1: rs1344410, Taq1: rs731236, and Fok1: rs10735810) have been investigated extensively in relation to cancer risk, particularly among European-ancestry populations (2,3). However, none of these SNPs were associated with breast cancer risk in our study. In a meta-analysis of 21 case-control studies (2) and in a pooling analysis of 6 prospective studies (3), breast cancer risk was associated with the VDR Fok1 polymorphism, however, the Cancer Prevention Study (CPS) II Nutrition Cohort in the United States did not find a relationship between any of these VDR SNPs and breast cancer risk among postmenopausal women (7). In addition, prior studies showed that CYP24A1 was overexpressed in breast carcinoma (8). In our study, we also found no associations of CYP24A1 polymorphisms with breast cancer risk, consistent with the CPS II Nutrition Cohort (7).

In a recent GWAS conducted among 5 European-ancestry cohorts in the United States, several SNPs in the GC, DHCR7/NADSYN1, and CYP2R1 genes and C10orf88 (in the vicinity of ACADSB) were associated with 25(OH)D level (4). Another GWAS [the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits (SUNLIGHT)] of 15 cohorts among 33,996 individuals of European ancestry found an association for 3 other SNPs in DHCR7/NADSYN1, GC, and CYP2R1 (5). None of these 25(OH)D-associated gene variants were related to breast cancer risk in our population.

Strengths of this study include its large sample size, population-based design, comprehensive analysis involving multiple genes, and control of potential confounding variables. A limitation of this study is that we have no direct measurements of circulating 25(OH)D level or vitamin D status. In addition, our analysis was conducted among Chinese women and the results may not be generalizable to other ethnic groups/populations.

In conclusion, this comprehensive survey provides no strong evidence for the hypothesis that genetic variants in a set of genes related to vitamin D level and metabolism play an independent role in breast cancer development among Chinese women.

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the NIH. The authors have no conflicts of interest to disclose.

Acknowledgments

The authors thank the participants and research staff of the Shanghai Breast Cancer Study for their contributions and commitment to this project and Bethanie Rammer for assistance with the preparation of the manuscript. Sample preparation and genotyping assays, using Affymetrix arrays, were conducted at the Survey and Biospecimen Shared Resource and the Vanderbilt Microarray Shared Resource, respectively.

Grant Support

This work was supported by USPHS grants R01CA064277, R01CA099099, and R01CA124558. Survey and Biospecimen Shared Resource and the Vanderbilt Microarray Shared Resource are supported in part by the Vanderbilt-Ingram Cancer Center (P30CA66485).

Received July 22, 2011; accepted July 28, 2011; published OnlineFirst August 8, 2011.

References

Common Genetic Variants in the Vitamin D Pathway Including Genome-Wide Associated Variants Are Not Associated with Breast Cancer Risk among Chinese Women

Tsogzolmaa Dorjgochoo, Ryan Delahanty, Wei Lu, et al.

Cancer Epidemiol Biomarkers Prev 2011;20:2313-2316. Published OnlineFirst August 9, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1055-9965.EPI-11-0704

Supplementary Material
Access the most recent supplemental material at:
http://cebp.aacrjournals.org/content/suppl/2011/08/09/1055-9965.EPI-11-0704.DC1

Cited articles
This article cites 8 articles, 3 of which you can access for free at:
http://cebp.aacrjournals.org/content/20/10/2313.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/20/10/2313.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.